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TRAINING PROGRAM FOR THE ANALYSIS OF DNA DATA BANK SAMPLES USING PCR-BASED STR FLUORESCENCE IMAGING ANALYSIS AT THE POWERPLEX® 16 BIO LOCI	Issue No. 1
	Effective Date: 1-August-2003
<p>5 AMPLIFICATION</p> <p>5.1 GOALS:</p> <p>5.1.1 To develop an understanding and working knowledge of the amplification process, including proper documentation.</p> <p>5.1.2 To become familiar with problems associated with amplification.</p> <p>5.1.3 To understand the importance of an amplifying environment that has no contamination.</p> <p>5.1.4 To understand the importance of quality control associated with the amplification process.</p> <p>5.2 TASKS:</p> <p>5.2.1 Work in an environment free of contamination and follow proper guidelines to prevent contamination.</p> <p>5.2.2 Program a thermal cycler and perform the quality control test on the thermal cycler</p> <p>5.2.3 Perform amplification using the PowerPlex® 16 BIO System. Refer to the <u>Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedures Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 1.1, 2.1, and 16 BIO Systems Manual</u> for the procedure.</p> <p>5.2.4 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A and B, and C for Additional Training Aids.</p> <p>5.2.5 Continue on to Chapter 6, PRODUCT GEL.</p> <p>5.3 TRAINING EVALUATION:</p> <p>5.3.1 Knowledge</p> <p>5.3.1.1 Review of notes and worksheets in training notebook by training coordinator.</p> <p>5.3.1.2 Oral and practical examinations.</p> <p>5.3.2 Skills</p> <p>5.3.2.1 The trainee should demonstrate an unquestionably sound technique for DNA amplification by consistently achieving uncontaminated results on the product gel. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.</p> <p>5.3.3 Completion of the checklist by training coordinator.</p>	

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<p>STUDY QUESTIONS:</p> <ol style="list-style-type: none"> 1. What is a nuclease? 2. What is an endonuclease? 3. What is an exonuclease? 4. Explain the amplification process 5. What is a DNA polymerase? 6. What is the name of the DNA polymerase that is use by the DFS and how does it work? Why do we use this DNA polymerase? 7. What is a primer? 8. What is the function of the primer? 9. What is the origin of the primer? 10. What is the function of MgCl₂? 11. What impact does the use of AmpliTaq Gold™ have on the PCR process? 12. What is primer - dimer? 13. How can primer - dimer affect the results? 14. Explain denaturation, annealing, and extension of the DNA? 15. What is preferential amplification (allelic drop out) and why does this occur? Is this a problem when analyzing samples using the STR technology? Why or why not? 16. What precautions are used to ensure that allelic drop out has not occurred? 17. What is plateau effect and how does it affect the DNA sample? 18. What are the components of the PowerPlex® 16 BIO reaction mix? What is the purpose of each component? 19. What are some of the factors that inhibit amplification and why? What steps can the analyst take to overcome inhibition problems? 20. What are the amplification conditions for the PowerPlex® 16 BIO System kit? 21. How many primers are used in the PowerPlex® 16 BIO System? 22. What precautions are used to prevent contamination of the sample DNA with a foreign source? Provide at least three different precautions/measures that are utilized in the laboratory. 	

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<p>23. What measures are taken to ensure that the thermal cycler is working properly? What is the purpose of each quality control test?</p>	

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CHECKLIST FOR AMPLIFICATION

- The trainee has demonstrated he/she can work in an environment free of contamination and follow proper guidelines to prevent contamination.

Date: _____ Training Coordinator: _____

Comments: _____
- Trainee has successfully programmed and performed QC on the thermal cycler.

Date: _____ Training Coordinator: _____

Comments: _____
- Trainee has successfully and accurately completed all appropriate paperwork associated with the amplification of the samples using the PowerPlex® 16 BIO System.

Date: _____ Training Coordinator: _____

Comments: _____
- Trainee has performed amplification on a minimum of 50 bloodstains and 150 buccal samples using the using the PowerPlex® 16 BIO System

Date: _____ Training Coordinator: _____

Comments: _____
- Trainee has developed an understanding of the theory of the amplification process, including the importance of quality control.

Date: _____ Training Coordinator: _____

Comments: _____
- Notebook is organized and complete.

Date: _____ Training Coordinator: _____

Comments: _____
- Trainee has read and understands all applicable literature.

Date: _____ Training Coordinator: _____

Comments: _____

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<p>8. Trainee has participated in question and answer sessions.</p> <p>Date:_____ Training Coordinator:_____</p> <p>Comments:_____</p> <p style="text-align: right;">◆END</p>	